

What is claimed is:

1. A method for amplifying a nucleic acid molecule, said method comprising
incubating an RNA template with a composition comprising (a) a buffer,
(b) two or more proteins having reverse transcriptase activity and (c) at least one DNA polymerase;
under conditions which substantially relieve reverse-transcriptase-mediated inhibition of DNA polymerase activity and which are sufficient to amplify a DNA molecule complementary to all or a portion of said RNA template.
2. A method according to claim 1, wherein said two or more proteins having reverse transcriptase activity comprise: a first reverse transcriptase enzyme in which the reverse transcriptase activity resides in a single polypeptide; and a second reverse transcriptase enzyme in which the reverse transcriptase activity resides in a dimeric or multimeric structure.
3. The method according to claim 2 wherein said first reverse transcriptase enzyme in which the reverse transcriptase activity resides in a single polypeptide is Moloney murine leukemia virus (M-MLV) reverse transcriptase or a derivative thereof having reduced RNase H activity and said second reverse transcriptase enzyme in which the reverse transcriptase activity resides in a dimeric or multimeric structure is AMV reverse transcriptase or a derivative thereof having reduced RNase H activity.
4. The method according to claim 1, wherein said composition comprises a first primer and a second primer,
wherein said first primer is suitable for facilitating synthesis of first strand cDNA from said RNA template, and wherein the combination of said first and said second primer is suitable for amplifying said first strand cDNA.
5. The method according to claim 1, wherein said buffer comprises an effective amount of at least one glutamate-containing compound.

6. The method according to claim 5, wherein the total glutamate concentration is about 1 mM to about 500 mM.

7. A method for accurately quantifying a nucleic acid molecule in an essentially sequence-independent manner, said method comprising

incubating an RNA template with a composition comprising (a) a buffer, (b) two or more proteins having reverse transcriptase activity, (c) at least one DNA polymerase, and (d) a first primer and a second primer,

wherein said first primer is suitable for facilitating synthesis of first strand cDNA from said RNA template, and wherein the combination of said first and said second primer is suitable for amplifying said first strand cDNA,

and wherein said incubation is under conditions which substantially relieve reverse-transcriptase-mediated inhibition of DNA polymerase activity and which are sufficient to amplify a DNA molecule complementary to all or a portion of said RNA template.

8. A method for the unbiased quantification of a nucleic acid molecule contained in a sample, said method comprising

incubating an RNA template with a composition comprising (a) a buffer, (b) two or more proteins having reverse transcriptase activity, (c) at least one DNA polymerase, and (d) a first primer and a second primer,

wherein said first primer is suitable for facilitating synthesis of first strand cDNA from said RNA template, and wherein the combination of said first and said second primer is suitable for amplifying said first strand cDNA,

and wherein said incubation is under conditions which substantially relieve reverse-transcriptase-mediated inhibition of DNA polymerase activity and which are sufficient to amplify a DNA molecule complementary to all or a portion of said RNA template.

9. A method according to claim 7, wherein said two or more proteins having reverse transcriptase activity comprise: a first reverse transcriptase enzyme in which the reverse transcriptase activity resides in a single polypeptide; and a second reverse transcriptase enzyme in which the reverse transcriptase activity resides in a dimeric or multimeric structure.

10. The method according to claim 9 wherein said first reverse transcriptase enzyme in which the reverse transcriptase activity resides in a single polypeptide is Moloney murine leukemia virus (M-MLV) reverse transcriptase or a derivative thereof having reduced RNase H activity and said second reverse transcriptase enzyme in which the reverse transcriptase activity resides in a dimeric or multimeric structure is AMV reverse transcriptase or a derivative thereof having reduced RNase H activity.

11. The method according to claim 8, wherein said two or more proteins having reverse transcriptase activity comprise: a first reverse transcriptase enzyme in which the reverse transcriptase activity resides in a single polypeptide; and a second reverse transcriptase enzyme in which the reverse transcriptase activity resides in a dimeric or multimeric structure.

12. The method according to claim 11 wherein said first reverse transcriptase enzyme in which the reverse transcriptase activity resides in a single polypeptide is Moloney murine leukemia virus (M-MLV) reverse transcriptase or a derivative thereof having reduced RNase H activity and said second reverse transcriptase enzyme in which the reverse transcriptase activity resides in a dimeric or multimeric structure is AMV reverse transcriptase or a derivative thereof having reduced RNase H activity.

13. The method according to claim 7, wherein said buffer comprises an effective amount of at least one glutamate-containing compound.

14. The method according to claim 8, wherein said buffer comprises an effective amount of at least one glutamate-containing compound.

15. The method according to claim 13, wherein said at least one glutamate compound is selected from the group consisting of glutamate salts of organic bases, alkali metal glutamate salts and alkaline earth metal glutamate salts.

16. The method according to claim 14, wherein said at least one glutamate compound is selected from the group consisting of glutamate salts of organic bases, alkali metal glutamate salts and alkaline earth metal glutamate salts.

17. The method according to claim 1, wherein said buffer further comprises an effective amount of an antifoam compound.

18. The method according to claim 5, wherein said buffer further comprises a sulfur-containing compound and a potassium-containing compound.

19. The method according to claim 1, wherein said DNA polymerase is a thermostable DNA polymerase.